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Association between NOS3 intron 4 VNTR variant and sports performance

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Abstract
Aim: Evidence shows the involvement of Nitric oxide (NO) in glucose metabolism, which is glucose uptake in human skeletal muscle at the time of exercise, controlling function and structure of skeletal muscle, conversion of skeletal muscle fiber type, production of mitochondrial ATP, and uptake of oxygen in skeletal muscles, all necessary for anaerobic and aerobic performance. Endothelial nitric oxide synthase (NOS3) enzyme synthesizes NO. The main purpose of this study was to investigate whether the NOS3 gene variable number of tandem repeats (VNTR) variant affected athletic performance in the Turkish population.
Material and Methods: This is a prospective single-center cross-sectional study. One hundred thirty-six Turkish athletes and 150 sedentary individuals as controls were included in the study. The intron-4 27-bp VNTR variant of NOS3 genotype and allele frequencies were analyzed with polymerase chain reaction (PCR) analysis.
Results: There was a significant difference between the athletes and the control group in the genotype distribution of the NOS3 VNTR variant. We found that the NOS3 VNTR 4a/4a genotype significantly increased in athletes compared with controls (X2:10.862, p = 0.004). A statistically significant association was observed between the athletes and the controls in terms of 4b/4b + 4a/4b genotype vs 4a/4a genotypes (p = 0.011, 95% CI: 0.0366 - 0.7915). There was no significant difference between the groups in the NOS3 VNTR allele distribution (p>0.05).
Discussion: This study gives evidence of an association between the NOS3 variant and sports performance. More studies should focus on the genetic effect of increased performance to confirm these findings.

Keywords
Nitric Oxide, ENOS, Sports Performance, VNTR, PCR

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Introduction

Study of sport sciences is faced with challenges of human athletic performance determinants. A significantly complex multifactorial phenomenon is sports performance, which is determined by various extrinsic factors such as nutrition, training, overall health conditions, development opportunities, and intrinsic factors such as psychological and physiological profile, motor behavior, genetics, and interaction [1]. As a gaseous free radical, nitric oxide (NO) functions as a multifunctional messenger [2]. Several studies show the involvement of NO in glucose metabolism, which is glucose uptake in human skeletal muscle at the time of exercise, controlling function and structure of skeletal muscle, conversion of skeletal muscle fiber type, production of mitochondrial ATP, and uptake of oxygen in skeletal muscles, all necessary for anaerobic and aerobic performance [3].

NO also helps repair and regenerate the myocardium and modulate consumption of oxygen in the myocardium [4]. Two NOS, i.e., nNOS and eNOS isoforms, have been found in skeletal muscle. The primary isoform found in skeletal muscle is nNOS, while eNOS, mainly expressed in endothelial cells, majorly helps control vascular tone. The NOS3 gene located on chromosome 7 (7q36) encodes NOS3 or eNOS as the endothelial nitric oxide synthase. The gene spans approximately 21 kb of genomic DNA and consists of 26 exons encoding a 1203 amino acids protein [5]. 4b/4a in intron 4, Glu298Asp (rs1799983) in exon 7, and T2786C (rs2070744) in the promoter region are regarded as polymorphisms found in the gene, which have been widely studied to see the relationship between the cardiovascular system functions in patients and healthy controls [6]. 4b/4a variant is placed in the minisatellite in intron 4 of the gene. Five VNTR alleles with 2–6 tandem 27-bp repeats (alleles 2–6) have been reported up to now. Alleles 4 and 5 are found in all investigated populations. Both genotype and allele frequencies of the VNTR show that there are interethnic variations [7]. Despite the lack of evidence on the exact molecular effects of this polymorphism, biochemical evidence shows the reduced expression of eNOS and enzymatic activity related to this NOS3 27-bp VNTR [6]. The intron 4 VNTR variant was reported to contribute to more than 25% of the phenotypic variation in the healthy Caucasian families with the observed NOS3 gene, where the minor allele a reduces the NO level [8].

Based on this data, the main purpose of this study was to investigate whether the NOS3 VNTR variant affected athletic performance in the Turkish population.

Material and Methods

Study population

The study group consisted of 136 athletes (34 females and 102 males) from different sports branches (football, basketball, volleyball, and wrestle) representing the Faculty of Sport Sciences, Ondokuz Mayıs University, Samsun, Turkey. The control group (43 females and 107 males) consisted of age-gender matched voluntary sedentary individuals studying at Ondokuz Mayıs University. The participants included both genders, were of Turkish descent and aged between 18 and 30 years. Informed written consent was obtained from all subjects before enrollment to the study, according to the Declaration of Helsinki's ethical guidelines, and the study was approved by the Ondokuz Mayıs University Ethics Committee (2020/45).

Genotyping

Two ml of venous blood was taken from each participant (athletes and controls), and DNA was extracted from all the samples using the commercial kit, based on the manufacturer's instructions. NOS3 VNTR variant was genotyped by the polymerase chain reaction (PCR) method described previously [9].

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows (SPSS Inc., Chicago, IL). The mean and standard deviation were used for the presentation of continuous quantitative variables. Frequencies and percentages were used for categorical data. The NOS3 overall genotype distribution was compared using the chi-square (x2) test, and the specific genotype and allele distributions were compared using Fisher's exact test. The odds ratios (ORs) and 95 % confidence intervals (CIs) were used to determine the relationships between the NOS3 allelic and genotypic variants and their occurrence in the patients. The p-values below 0.05 were considered statistically significant.

Results

In the present study, 286 subjects, including 136 athletes and 150 controls, were genotyped for the NOS3 VNTR variant. The baseline demographic features of the subjects are shown in Table 1.

In Table 2, the descriptive information of the participants according to some variables is given.

The distribution of genotypes and allele frequencies of NOS3 VNTR between athletes and controls is shown in Table 3. There was a significant difference between the athletes and controls in terms of the intron 4 VNTR variant of the NOS3

Table 1. Baseline demographic and clinical features of the subjects

		Control n=150	Athlete n=136
Female/Male (n.n)		43/107(%28.7/71.3)	34/102 (%25/75)
Age (years)		22.48±3.00	22.01±2.86
min-max		18-30	18-30
Weight (kg)	40-49	0	1 (%0.7)
	50-59	17 (%11.3)	20 (%14.7)
	60-69	39 (%26)	37 (%27.7)
	70-79	46 (%30.6)	41 (%30.1)
	80-89	31 (%20.6)	21 (%15.4)
	90-99	16 (%10.6)	13 (%9.6)
	100-109	1 (%0.66)	3 (%2.2)
Height (cm)	150-159	4 (%2.6)	5 (%3.7)
	160-169	22 (%14.6)	22 (%16.2)
	170-179	71 (%47.3)	59 (%43.4)
	180-189	42 (%28)	29 (%21.3)
	190-200	11 (%7.3)	21 (%15.4)
BMI	Lower than 18.5	1 (%0.66)	1 (%0.7)
	18.5 up to 25	109 (%72.6)	112 (%82.4)
	25 up to 30	39 (%26)	21 (%15.4)
	30 upwards	1 (%0.6)	2 (%1.5)

BMI:body mass index

gene. The NOS3 4b/4a genotype frequencies in elite athletes were 58.1%, 34.5%, and 7.4% for 4b/4b, 4b/4a, and 4a/4a, respectively, and 49.3%, 49.3%, and 1.4% in the control group for 4a/4a, 4a/4b, and 4b/4b, respectively. Genotype frequencies were significantly different between the groups (x2=10.862, p=0.004). A statistically significant association was observed between the athletes and the controls in terms of 4b/4b + 4a/4b genotype vs 4a/4a genotypes (p = 0.01122, 95% CI: 0.03663 - 0.7915). We found no significant difference between the athletes and the controls in terms of the allelic frequencies of the NOS3 intron 4 VNTR variant.

Table 2. Baseline clinical features of the subjects

		Control n=150	Athlete n=136
Smoking (day)	0	106 (%70.6)	109 (%80.1)
	5.9	6 (%4)	8 (%5.9)
	10.15	38 (%25.3)	19 (%14)
Alcohol Consumption (month)	0	122 (%81.3)	119 (%87.5)
	1.4	0	1 (%0.7)
	5.9	8 (%5.3)	4 (%2.9)
	10-14	12 (%8)	6 (%4.4)
	15-20	8 (%5.3)	6 (%4.4)
Sports Branch	Football	-	38 (%27.9)
	Basketball		36 (%26.5)
	Volleyball		43 (%31.6)
	Wrestle		19 (%14)
Training (weak)	2	-	1 (%0.7)
	3		45 (%33.1)
	4		50 (%36.8)
	5		40 (%29.4)
Family History	No	139 (%92.6)	86 (%63.2)
	Football	8 (%5.3)	15 (%11)
	Basketball	3 (%2)	4 (%2.9)
	Volleyball	0	5 (%3.7)
	Wrestle	0	19 (%14)
	Judo	0	5 (%3.7)
	Athletics	0	1 (%0.7)
	Swimming	0	1 (%0.7)
Disease	No	135 (%90)	121 (%89)
	Chronic Disease	15 (%10)	-
	Asthma		1 (%0.7)
	Chronic pharyngitis		1 (%0.7)
	Cross-link scission		3 (%2.2)
	Gastritis		1 (%0.7)
	Groin swelling		1 (%0.7)
	Hepatitis B		2 (%1.5)
	Meniscus		3 (%2.2)
	Migraine		1 (%0.7)
	Muscle Strain		1 (%0.7)
	Ulcerative colitis		1 (%0.7)

Discussion

In this study on Turkish athletes and controls, an association was found between eNOS gene intron 4b/4a VNTR polymorphism and sports performance. The main finding in our study overrepresented the 4a/4a genotype among the Turkish athletes as compared with the ethnically-matched controls. A complex phenotype affected by several genetic and environmental factors is physical exercise. Adaptations for the production of coordinated movements involve changes in cells and tissue, which convey the expression of the gene. For example, skeletal muscles can have varying efficiency, and the cardiovascular system can be almost exposed to fatigue, which depends on the expression of genes. NO is produced by the endothelium as the most potent vasodilator, increasing physical activity and induces shear stress. This is modulated by a genetic predispositions to the expression of NOS3 [10]. NO is a potent vasodilator while helping control skeletal muscle glucose uptake at the time of exercise (3), mitochondrial ATP production (3), and skeletal muscle function (3), all modulating strength muscle. NO is also produced in the cerebral circulation, affecting neuronal activity, including dopamine release [11]. Thus, actions mediated by NO also affect cognition and behavior, motivation, and voluntary movement. The NOS3 gene explains individual variations in phenotypes related to health and exercise capacity as it encodes NOS3. NOS3 is the enzyme catalyzing the NO synthesis. This is a well-known molecule helping regulate important body functions and systems [12]. Since NO plays a potential role in regulating tolerance to physical exercise and recovery mechanisms, testing the NOS3 genetic variants has shown its association with different exercise or training response phenotypes such as cardiovascular hemodynamics features such as heart rate responses [13] and blood pressure [14]. NO directly regulates basal vascular tone and helps supply blood to tissues, including potentially working muscles, while contributing to myocardial respiration and cardioprotection [15]. Based on different candidate gene studies, there is an association between polymorphisms of NOS3 gene -786T/C, G894T and VNTR and several training, health/fitness, or

Table 1. Genotype and allele frequencies of NOS3 VNTR in subjects

	Genotypes/Alleles	Athlete group	Control group	X^2	P	OR
		n=136 (%)	n=150 (%)			(CI 95%)
NOS3 VNTR	4b4b	79 (58.1)	74 (49.3)	10.862	0.004	
	4a4b	47 (34.6)	74 (49.3)			
	4a4a	10 (7.4)	2 (1.3)			
	4b4b + 4a4b: 4a4a	126:10	148:02	6.43	0.011	0.037-0.792
	4b4b: 4a4b + 4a4a	79:57	74:76	2.198	0.138	0.892-2.272
	4b	205	222	0.141	0.707	0.737-1.568
	4a	67	78			

exercise response phenotypes such as adapting heart rate responses [13], parasympathetic modulation response [16], cardiovascular traits such as athletes' cardio-biochemical [17], aerobic capacity, and blood pressure [18].

Eynon et al. found that genotype distribution of NOS3 -786 T/C significantly differed between soccer players, controls, endurance, and power elite athletes [19]. Also, it was shown that the frequency of the NOS3 -786 T/C TT genotype was significantly higher in power athletes than that in the endurance or control group [12]. Drozdovs'ka et al. observed that NOS3 -786 T/C T-allele frequency was higher in athletes in speed and power sports than in the control group and group endurance sports [17]. Eider et al. (2014) reported a significant difference between control subjects and the power-oriented, mixed, endurance athletes in NOS3 G894T genotype and allele distributions [20]. Zmijewski et al. (2018) demonstrate the benefit of the G-T haplotype and T allele of the G894T and -786T/C polymorphisms for long-distance swimmers [21]. NOS3 promoter activity can be affected by the 27-bp VNTR as a cis-acting factor, inhibiting the expression of NOS3. Sparse evidence shows the impact of this polymorphism on the expression and activity of NOS3 [22]. Endothelial cells with the 4-repeat (4a) allele increase NOS3 mRNA level compared to the cells with the more common 5-repeat (4b) allele [23]. It has been reported that the "4a" allele is associated with some disorders [24, 25].

This study examined the NOS3 VNTR genotype distribution among 150 control individuals and 136 athletes. This is the first study to evaluate the association between the NOS3 VNTR variant and athletic performance in Turkey to the best of our knowledge. We observed that the NOS3 VNTR 4a/4a genotype was higher in athletes than in the controls (Table 3). In our study, the 4a/4a genotype in the athletes was 10.862 times as much as that in the controls. This has been interpreted as a contribution to predisposition. In addition, there was a significant difference between the athletes and the controls in terms of 4b/4b + 4a/4b genotype vs. 4a/4a genotypes.

However, the present case-control analysis has several limitations. First, our focus was on only one variant in the NOS3 pathway, and the mechanism may be affected by other regulatory genes in this family signaling pathway.Second, since the sample size was relatively small, the groups had low frequencies of some homozygous variants, reducing the statistical power. Third, individuals of the same race and geographical region were sampled.

Conclusıon

This study gives evidence of an association between the NOS3 variant and sports performance. More studies should focus on the genetic effect of increased performance to confirm these findings.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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